A system for observing the behaviors of mitochondria during ontogeny and the dynamics of mitochondria in various tissues during pathological development is provided. A pCAGGS expression vector containing green fluorescent protein (GFP) gene and a transgenic animal characterized by expressing GFP specifically in mitochondria are provided.

6 Claims, 5 Drawing Sheets
(4 of 5 Drawing Sheet(s) Filed in Color)
Fig. 2

M B6 TG #20 #40 #49

258 bp

Fig. 3

Forelimbs of TG and WT mice immediately after birth observed under a fluorescent microscope
Fig. 4

![Fluorescence and Bright field images of TG and WT testis](image)

Fig. 5

![GFP, Mitotracker, and overlay images of Tg mouse-derived fibroblasts](image)

CLSM images of Tg mouse-derived fibroblasts
Fig. 6

- Before fertilization

- Immediately after fertilization

- Pronuclear stage

- 2-cell-stage

- 2-cell-stage

- 4-cell-stage
Fig. 7
1 GFP expression vector localized in mitochondria

The following application is a 371 of PCT/JP01/08585 which claims priority form JAPAN 2001-97102.

FIELD OF THE INVENTION

The present invention relates to a mitochondria-localized GFP expression vector, by which an introduced green fluorescent protein (GFP) gene is expressed locally in mitochondria, and a transgenic animal that expresses GFP specifically in mitochondria.

BACKGROUND OF THE INVENTION

Mitochondrial DNA (mtDNA) is known to show strict maternal inheritance in mammals. We have previously found that sperm mtDNA was eliminated from the cytoplasm of embryonic cells by the 2-cell-stage in early embryogenesis, using highly sensitive PCR methods (Kaneda, H. et al., (1995) Proc. Natl. Acad. Sci. USA 92, 4542-4546; and Shihara, H. et al., (1998) Genetics 148, 851-857). This phenomenon could cause the maternal inheritance of mtDNA.


However, several drawbacks are known regarding the above methods. The above dyes are easily bleached under the irradiation of UV light, and even without irradiation of UV light the dyes rapidly become bleached. The procedures involved in the staining such as centrifugation and resuspension damaged cells and mitochondria, and consequently the fertility of sperm is remarkably reduced, and the dyes are easily released after staining. These drawbacks make it difficult to conduct sequential or repetitive observations of mitochondria.

Instead of vital staining dyes, the use of green fluorescent protein (GFP), which has been widely applied as a non-invasive chemiluminescent reporter molecule, enables the visualization of the localization and/or migration of proteins of interest in intracellular compartmentation. GFP can also be used for real-time visualization of intracellular organelles. In particular, Rizzuto et al. (Rizzuto, R. et al., (1995) Curr. Biol. 5, 635-642; and Rizzuto, R. et al., (1996) Curr. Biol. 6, 183-188) reported that GFP was accumulated exclusively in the mitochondria of HeLa cells by transfection of a GFP DNA clone linked with a polynucleotide encoding the N-terminus of the mitochondrial cytochrome c oxidase subunit VII precursor protein (mGFP). Fluorescence was observed as a rod-like shape typical of mitochondria, suggesting that GFP is accumulated in mitochondria.

Moreover, the reduction of GFP fluorescence was not at all or less observed by repeated UV-irradiation, whereas the fluorescence of rhodamine 123, a vital staining dye specific to mitochondria, was dramatically reduced under the same conditions.

As described above, it has been expected that GFP linked with an importing signal to a certain organelle has wide applications in real-time observation of the intracellular organelle.

However, the above methods have drawbacks in that applicable cells are limited to cells such as established cultured cells and cells of tissue that can be subjected to primary culture, and that the behavior of mitochondria during ontogenesis and the dynamics of mitochondria in lesions during pathological development cannot be observed.

To express mitochondria-localized GFP in every tissue in an individual animal, an appropriate vector must be selected. To date, expression vectors having promoter sequences to enable tissue-specific expression or vectors that enable expression in all the tissues have been invented and improved. However, there are only few expression vectors that are completely sufficient, particularly in terms of the expression level of a foreign gene.

In the meantime, apoptosis, which is also referred to as programmed cell death and by which the cell content is not released outside the cell, in contrast to necrosis by which the cell content is released extracellularly, is known to cause disorders of mitochondria involving characteristic morphological changes such as the aggregation or fragmentation of nuclei within cells and the discharge of cytochrome C. Recently, the involvement of apoptosis in ontogeny and various diseases is increasingly reported. However, most of the facts about apoptosis remain unknown.

SUMMARY OF THE INVENTION

As a result of a variety of studies undertaken to solve the above problems, we have selected a pcAGGS expression vector, which enables strong expression of a foreign gene in every tissue. We have further constructed a mitochondria-localized GFP expression vector to label mitochondria using GFP, and generated as well a transgenic (Tg) animal having mitochondria visualized by GFP.

That is, the present invention provides the following (1) to (10):

(1) A mitochondria-localized GFP expression vector, which is a pcAGGS expression vector containing a green fluorescent protein (GFP) gene, wherein the GFP is locally expressed in mitochondria.

(2) The expression vector of (1) above, which has an importing signal sequence to mitochondria.

(3) The expression vector of (2) above, wherein the importing signal sequence is the signal sequence of cytochrome c oxidase subunit VIII.

(4) A mitochondria-localized GFP expression vector, which is an expression vector containing a GFP gene, wherein the GFP is locally expressed in mitochondria.

(5) A transgenic animal, wherein the expression vector of any one of (1) to (4) above has been introduced.

(6) A transgenic animal, wherein GFP is expressed specifically in mitochondria.

(7) The transgenic animal of (6) above, wherein a gene for an importing signal to mitochondria and a GFP gene have been introduced.

(8) A cell having the expression vector of any one of (1) to (4) above introduced.

(9) A tissue or cell derived from the transgenic animal of any one of (5) to (7) above.

(10) A screening method for an inducer of a disorder of mitochondria or apoptosis, comprising the steps of:
3 (a) contacting the transgenic animal of any one of (5) to (7) above or the cell of (8) or (9) above with a test substance; and
(b) confirming whether or not a disorder of mitochondria or apoptosis is induced.
(H) A screening method for an apoptosis suppressor, comprising the steps of:
(a) contacting the transgenic animal of any one of (5) to (7) above, or the cell of (8) or (9) above with a test substance;
(b) treating the above animal or the above cell with the apoptosis inducer; and
(c) confirming whether or not apoptosis is suppressed.

The present invention will be described in detail as follows.

The green fluorescent protein (GFP) used in the present invention may be any protein that is used in the art. A genetically modified GFP having enhanced fluorescence intensity or having fluorescence that disappears within a certain period of time can also be used. Examples of a commercially available GFP product include pEGFP and pEGFP-N1/2/3 (CLONTECH, cat. #6077, K6001-1). As described above, GFP has the excellent features that it is noninvasive and that it is not bleached by UV irradiation or the like.

Any vector that is used in the art can be appropriately used as an expression vector that can be used in the present invention. Specifically, the expression vector may be selected according to the types, etc. of cells or animals for the above GFP gene to be introduced, and is not specifically limited. The pCA326 expression vector (Niwa, B. et al., 1991) Gene 108, 193–200), which has no tissue specificity and by which strong expression can be expected, can be preferably used. Further, when a gene is introduced into a cell or an animal, the expression vector may be introduced intact, or a target gene may also be introduced after preparation of an expression cassette containing the target gene. The introduction method may be appropriately selected according to the cell or animal to which a gene is to be introduced. Therefore, the “expression vector” in the present specification also includes the above expression cassette.

For the introduced GFP to be expressed locally and specifically in mitochondria, for example, an expression vector preferably has an importing signal sequence to mitochondria on the upstream side of the GFP gene. Examples of the importing signal sequence include that of cytochrome c oxidase subunit VIII, and cDNA of cytochrome C. The GFP gene and the above importing signal sequence may be adjacent to each other or separated from each other by several tens of nucleotides, but the reading frames must be the same.

In addition, the expression vector of the present invention may contain regulatory sequences such as a promoter and a terminator, and a tag such as HA1 for appropriately regulating the expression of the gene to be introduced.

The transgenic animal of the present invention is characterized in that GFP is specifically expressed in mitochondria, and in particular the above expression vector has been introduced.

Examples of the types of animals used herein may be any animals other than humans, and include, but are not limited to, mammals such as a mouse, rat, hamster, guinea pig, rabbit, pig, miniature pig, cattle, sheep, cat and dog, birds such as a chicken, fish, insects such as drosophila and nematodes. In the present invention, the animal is preferably a rodent, and particularly preferably a mouse in terms of feeding and operation.

For example, the transgenic animal of the present invention can be generated as described below. First, an expression vector is constructed as described above, an expression cassette is excised from the expression vector by cleavage using restriction enzymes or the like, and then the expression cassette is introduced into totipotent cells. Examples of totipotent cells that can be used herein include fertilized eggs, early embryos and embryonic stem cells (ES cells). Introduction into totipotent cells can be performed by techniques that are normally employed in the art such as an electrostatic pulse method, a liposome method, a calcium phosphate method or a microinjection method. The above-treated totipotent cells are transplanted in the oviduct of a pseudo-parent to produce progenies. Then animals having GFP gene are selected from the progenies. Whether or not animals have GFP gene can be confirmed by any conventional method such as Southern blotting or PCR using a GFP gene-specific probe or primers. In the present invention, it can also be determined by visually observing the expressed GFP.

The present invention also provides a cell, wherein the above mitochondria-localized GFP expression vector has been introduced. The cells of the present invention are not limited to those derived from the above transgenic animal, and may be any cells of eukaryotes having mitochondria. Examples of the cell include those of a plant, yeast, and drosophila. The expression vector of the present invention can be introduced into these cells by techniques that are normally employed in the art.

Fluorescence originating from GFP is localized in the mitochondria of the transgenic animal, tissue and cells of the present invention and is specifically expressed. Hence, the use of the transgenic animal of the present invention enables noninvasive observation of, for example, the state of sperm mitochondria during early embryogenesis. Fluorescence of the transgenic animal of the present invention is strong and stable, so that repeated observation under a confocal laser scanning microscope can be conducted.

As described above, GFP expression is localized in mitochondria of the transgenic animal according to the present invention. However, the localized area is changed to the whole cytoplasm by treatment with an inducer of a disorder of mitochondria, apoptosis or the like. We have confirmed that the GFP localization pattern is changed by the inducer of a disorder of mitochondria or apoptosis, using cells obtained from the transgenic animal of the present invention. Specifically, the determination of GFP localization using the transgenic animal or the cells of the present invention makes it possible to easily determine whether or not apoptosis occurs. For example, the transgenic animal of the present invention can be used as a model animal to study the mechanism, etc. of apoptosis.

Moreover, there are many known cases where the involvement of a disorder of mitochondria (including apoptosis) in the pathological development and progress of various diseases is assumed. Thus, various pathological models (brain ischemia model, cardiac muscle ischemia model and the like) are prepared using the transgenic animal obtained by the present invention, so as to be able to know the sites and the degrees of disorders of mitochondria in various pathological conditions, elucidate the pathological conditions and characterize the models. Further, the use of the transgenic animal according to the present invention or cells derived from the transgenic animal enables screening for suppressors, inducers or the like of disorders of mitochondria.
Specifically, a test substance is allowed to come into contact with the transgenic animal or the cell of the present invention to confirm whether or not a disorder of mitochondria or apoptosis is induced, so that screening for an inducer of the disorder of mitochondria or apoptosis can be performed.

Moreover, using a conventionally known apoptosis inducer such as staurosporine, a Fas antibody or an apoptosis inducer obtained by the above screening, an apoptosis suppressor can also be screened for by contacting a test substance with the transgenic animal or the cell of the present invention, and then treating with the apoptosis inducer to confirm whether or not apoptosis has been suppressed.

By the use of the transgenic animal or the cell of the present invention, the induction and the suppression of apoptosis can be easily known visually due to the fluorescence of GFP. In the above screening method, to confirm the induction or the suppression of apoptosis, preferably, a comparison with a control animal or a control cell under the same conditions except for a lack of contact with the above test substance may be performed. Alternatively a comparison with the states before the contact with the test substance or the state before the treatment using an apoptosis inducer are preferably performed.

In the above screening method, the test substance is not specifically limited. Examples of the test substance include a peptide, a protein, a non-peptide compound, a synthetic compound, a fermentation product, and a cell extract.

In the above screening method, a test substance may be caused to come into contact in vitro when the cells are used. When the transgenic animal is used, any conventionally known method of local or systemic administration may be employed, including oral administration or parenteral administration such as subcutaneous injection or intraperitoneal injection. Such the administration methods can be appropriately selected according to the types of animals used, pathological conditions, the presence or the absence of target tissue and the like.

The apoptosis inducer obtained by the above screening method can be used for, for example, therapies against cell proliferative diseases such as cancer. In addition, the apoptosis suppressor obtained by the above screening method can be used for, for example, therapies against diseases caused by cell death such as Alzheimer’s disease or ischemic disease.

**BRIEF DESCRIPTION OF THE DRAWINGS**

The patent or application file contains at least one drawing executed in color. Copies of this patent or patent application publication with color drawings will be provided by the Office upon request and payment of the necessary fee.

FIG. 2 is a photograph showing the detection of a transgene by the PCR method.

FIG. 3 shows photographs showing GFP expression in the transgenic mouse.

FIG. 4 shows photographs showing GFP expression in the tissue of the transgenic mouse.

FIG. 5 shows photographs showing GFP expression in the cells of the transgenic mouse.

FIG. 6 shows photographs showing the detection of sperm-derived mitochondria in the process of early mouse development.

FIG. 7 shows photographs showing the dynamics of mitochondria in mouse fibroblasts on apoptosis induction.

The patent or application file contains at least one drawing executed in color. Copies of this patent or patent application publication with color drawings will be provided by the Office upon request and payment of the necessary fee.

**BEST MODE OF CARRYING OUT THE INVENTION**

**EXAMPLE 1**

**Construction of Expression Vector**

The signal sequence of cytochrome c oxidase subunit VIII (Cox8, Genbank U15541) was amplified by PCR reaction using primers, 5'-GCA GAA TCC TGC ACC ACC ATG CCA AGG CTC CCC CC-3' (SEQ ID NO: 1) and 5'-GGC GGA TCC TAA GCT TGC ATA ATG AGG AAC ATC ATA ATG GGC TTT GGG AAC C-3' (SEQ ID NO: 2) and C57BL/6J(b6)-derived genomic DNA as a template. In this case, the primer of SEQ ID NO: 2 contained the nucleotide sequence of HA1 (TAA GCT TGC ATA ATG AGG AAC ATC ATA) plus the deduced nucleotide 30 to 36 of SEQ ID NO: 2). Therefore, the HA1 nucleotide sequence was incorporated in the amplification product obtained by the PCR reaction. The coding region of EGF (SEQ ID NO: 10) was amplified by PCR reaction using primers, 5'-GAT GGA TCC ATC GGC ACC ATG GTG AGC AAG-3' (SEQ ID NO: 3) and 5'-GGG AAT TCT TAC TTG TAG ACG ATC TGC TTC ATC CG-3' (SEQ ID NO: 4) and a recombinant plasmid pEGFP-N3 (Clontech, Calif.) as a template. Both PCR products were cleaved with EcoR I and BamH I and ligated into a pCAGGS expression vector (Niwa, B. et al., (1991) Gene 108, 193–200) that was already cleaved with EcoR I.

The structure of the main portion of the expression vector constructed by the above procedures is shown in FIG. 1, and the entire sequence is shown in SEQ ID NO: 9.

**EXAMPLE 2**

**Generation of Transgenic Mouse**

DNA fragments to be used for the generation of a transgenic animal were obtained by double digestion using Sal I and Stu I (FIG. 1), separated by agarose gel electrophoresis from the cloning vector, and then purified by QIAEX II (QIAGEN, Calif.). The purified DNA fragments were inserted into the pronuclei of fertilized mouse (b6) oocytes according to the standard procedure (Hogan, B. et al., (1994) Manipulating the Mouse Embryo 2nd edn., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.). To identify transgenic mice, genomic DNA was prepared from ear-punched pieces. Specifically, the punched pieces were incubated overnight at 37° C. in a PCR buffer/nonionic surfactant and proteinase K (50 mM KCl, 10 mM Tris-HCl, pH 8.5, 1.5 mM MgCl2, 0.1% gelatine, 0.45% NP-40, 0.45% Tween-20 and 100 µg/ml proteinase K). To inactivate protein kinases, incubation was performed at 95° C. for 15 minutes. The obtained solution was used as a DNA sample.

Next, a PCR fragment (258 bp) specific for the transgene sequence was amplified using the prepared genomic DNA as a template and 2 sets of synthesized primer pairs (A) 5'-GCT CTA GAG CCT CTC GAA C-3' (SEQ ID NO: 5) and
Confirmation of GFP Expression in Individual Transgenic Mouse

To confirm GFP expression in the transgenic mice obtained in Example 2, the forelimbs of an individual mouse were observed immediately after birth under a fluorescence microscope.

Fluorescence of EGFP was visualized under a Carl Zeiss laser scanning confocal microscope (LSM510). EGFP fluorescence was excited with a 488 nm argon-ion laser and imaged through a 505-550 nm bandpass emission filter.

As shown in Fig. 3, fluorescence resulting from GFP expression was observed throughout the forelimbs of the transgenic mouse (Tg) of the present invention. The contours could be distinctly observed as clearly recognized by a comparison with a photograph in a bright field. In contrast, fluorescence was not observed at all for the wild type (WT).

Confirmation of GFP Expression in the Tissue of the Transgenic Mouse

Mouse testes were isolated from 12-week-old mtGFP-Tg mice, frozen with liquid nitrogen, and then stored in a deep freezer (−80°C). To prepare 10 μm-thick sections, the frozen testes were sliced using a cryostat at −20°C and then placed on a slide glass. Fluorescence was observed using a fluorescence microscope.

As shown in Fig. 4, fluorescence resulting from GFP expression was observed for the transgenic mouse of the present invention. The contours could be distinctly observed as clearly recognized by comparison with a photograph taken in a bright field. In contrast, fluorescence was not observed at all for the wild type (WT).

Confirmation of GFP Expression in the Fibroblasts of Transgenic Mouse

Primary culture of fibroblasts was performed according to the Explant Culture Method (Fischer SM et al., Methods in Cell Biology (edited by Harris, C. C. et al.) (1980) 21: 207-227, Academic Press, London). The cultured fibroblasts were incubated in DMEM containing 500 mM Mitotracker Red CMXRos (Molecular Probes, OR; cat. M7512) for 15 minutes at 37°C. Then, the stained fibroblasts were washed twice with DMEM and observed using a confocal laser microscope.

Fluorescence of EGFP or Mitotracker Red CMXRos was visualized under a Carl Zeiss laser scanning confocal microscope (LSM510). EGFP fluorescence was excited with a 488 nm argon-ion laser and imaged through a 505-550 nm bandpass emission filter. Mitotracker Red CMXRos fluorescence was excited with a 543 nm HeNe laser and imaged through a 560 nm longpass emission filter.

As shown in Fig. 5, the images by GFP fluorescence and Mitotracker fluorescence almost corresponded to each other.

Trend in the Early Development Process of Sperm-derived Mitochondria

Sperms, eggs and embryos were collected as we previously described (Kaneda, H. et al., (1995) Proc. Natl. Acad. Sci. U.S.A. 92, 11331–11338). Sperms were prepared as a sperm suspension by extracting the cauda epididymis from adult mice, and then adding the extract to a TYH medium (Toyota, H. et al., (1971) Jpn J. Anim. Reprod. 16, 147–151). Unfertilized eggs were collected from the oviducts of female mice superovulated by pregnant mare's serum gonadotropin and human chorionic gonadotropin (Hogan, B. et al., (1986) Manipulating the Mouse Embryo: A Laboratory Manual (Cold Spring Harbor Lab. Press, Plainview, N.Y.). Early embryos were collected from the oviducts or uteri of naturally mated mice. A single sperm or a single egg was isolated from the suspension of the sperms or eggs that had been diluted using a capillary for microinjection under a phase contrast microscope, put into 10 μl of distilled water, and then stored at −80°C.

Fig. 6 shows the detection of sperm-derived mitochondria in the early development process of mouse. "a" and "b" show a sperm and an egg before fertilization. The mitochondria in the middle piece of the sperm were observed as green fluorescence (c: fluorescence and b: bright field). "c (fluorescence)" and "d (bright field)" show an embryo immediately after fertilization. "e (fluorescence)" and "f (bright field)" show a pronuclear-stage embryo. "g (fluorescence)" and "h (bright field)" show a two-cell-stage embryo, and fluorescence of sperm-derived mitochondria was not observed. However, an embryo in which fluorescence remained was observed although at a low rate (i: fluorescence, and j: bright field). "k (fluorescence)" and "l (bright field)" show a four-cell-stage embryo, in which GFP was expressed by the male (sperm)-derived transgene.

Apoptosis Induction in Mouse Fibroblasts and Observation Thereof

1) Preparation of Cells
Using abdominal skin sections of generated transgenic mice neonates (mtGFP-Tg mice), culture of fibroblasts was begun according to the explant culture method as employed in Example 5. First, the abdominal skin sections of the neonates were cut into approximately 1 mm square, placed on a plastic dish (FALCONE 3001), and then incubated under conditions of 37°C and 5% CO2 for 15 to 30 minutes. 10% NCS-DMEM (GIBCO, without phenol red) was then added. On days 7 to 10 after the start of culture, cells were stripped using 0.25% trypsin, and then centrifuged at 700 to 800 g at 4°C for 5 minutes. The precipitate was resuspended in 10% NCS-DMEM, and then inoculated onto a cover glass (Muranami). One day later, the cells were used for the experiment of apoptosis induction.

2) Apoptosis Induction Method
The medium was exchanged with a medium containing 10% NCS-DMEM and 5 μM staurosporine, the apoptosis inducer.

3 to 5 hours later, cells were observed using a fluorescence microscope (Zeiss, Axiosphoto; FITC filter set). In addition, Hoechst 33342, the fluorescent DNA staining reagent (with a final concentration of 200 μM) was added, and then the state of the nuclei was observed. The transgenic mouse-derived fibroblasts were observed before (0 hr) and after (2.5 hr) the addition of staurosporine
(FIG. 7). Under a phase contrast microscope, it was observed that the cell slightly changed morphologically after the addition of staurosporine. Further when the morphology of the cell nuclei was observed by Hoechst 33342 staining, chromatin aggregation that is characteristic of cell nuclei undergoing apoptosis was observed. At this time, GFP-labeled mitochondria were observed as existing uniformly in the cytoplasm.

Sequence Listing Free Text
SEQ ID NO: 3: Primer for PCR
SEQ ID NO: 4: Primer for PCR
SEQ ID NO: 5: Primer for PCR
SEQ ID NO: 6: Primer for PCR
SEQ ID NO: 7: Primer for PCR
SEQ ID NO: 8: Primer for PCR
SEQ ID NO: 9: Expression vector pCAGGS-COX8-EGFP
SEQ ID NO: 10: Aequorea-derived EGF coding region modified for codon optimization

INDUSTRIAL APPLICABILITY
The transgenic animal obtained by the present invention expresses GFP, which is observable with its fluorescence, specifically in mitochondria, so that it enables visual capturing of the trend of mitochondria in cells, tissue and animals without any complicated staining process. Further, according to the invention, the degree of color fading is significantly lower than that of dyes which have been conventionally used, enabling sequential observation that has conventionally been impossible. Furthermore, the transgenic animal of the present invention expresses GFP throughout the body, so that it can be used as a supply source of various cells having mitochondria-localized GFP.

Moreover, the present invention also makes it possible to conduct various analyses regarding the localization of mitochondria in each of the tissues and cells of, for example, a pathological model animal, the trend of sperm-derived mitochondria in the early development process, and the dynamics of mitochondria during the apoptosis process.

Further, according to the screening method using the transgenic animal or the cells of the present invention, there is provided a novel method for simply screening for an apoptosis inducer and suppressor. As described above, the present invention has an extremely wide applicable range in the fields of molecular biology and medicine.

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Ser Lys Gly Glu Glu Leu Phe Thr Gly Val Val Pro Ile Leu Val Glu
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ctg gac ggc gac gta aac ggc cac aag ttc acc ggc gta gtt ggc gag ggc
Leu Asp Gly Asp Val Asn Gly His Phe Ser Val Ser Gly Glu Glu
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gag ggc gat gac acc tac ggc aag ctc ctc aag ctc aag ctc ctc acc ggc ggc
Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr Leu Lys Phe Ile Cys Thr
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Thr Gly Lys Leu Pro Val Val Pro Ile Thr Thr Tyr Val Thr Thr Leu Thr
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tat ggc gtc cag tgc ctc acc ggc tac ctc ctc ctc ctc ctc ctc ctc ctc
Tyro Glu Val Gin Cys Phe Ser Arg Tyr Pro Asp His Met Lys Gin His
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gac ttc aag ttc ggc atg ggc cag ggc tac gtc gca ggc acc
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85 90 95
| ATC   | TCC   | AAG   | GAC   | GAC   | GAC   | AAC   | AAG   | CCG   | GAC   | GAG   | GTG   | AAG   | GAC   | GAG   | GTG   | AAG   | GAC   | GAG   | GTG   | AAG   | GAC   | GAG   | GTG   | AAG   | GAC   | GAG   | GTG   | AAG   | GAC   | GAG   | GTG   | AAG   | GAC   | GAG   | GTG   | AAG   | GAC   | GAG   | GTG   | AAG   | GAC   | GAG   | GTG   | AAG   | GAC   | GAG   | GTG   | AAG   | GAC   | GAG   | GTG   | AAG   | GAC   | GAG   | GTG   | AAG   | GAC   | GAG   | GTG   | AAG   | GAC   | GAG   | GTG   | AAG   | GAC   | GAG   | GTG   | AAG   | GAC   | GAG   | GTG   | AAG   | GAC   | GAG   | GTG   |
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|       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |�

**US 7,227,052 B2**
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<223> OTHER INFORMATION: Description of Artificial Sequence: Aequorea victoria SOFP coding region modified for codon optimisation

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20    25    30

Glu Gly Glu Asp Ala Thr Tyr Gly Lys Leu Thr Leu Lys Phe Ile
35    40    45
What is claimed is:

1. A transgenic mouse whose genome comprises a green fluorescent protein (GFP) gene operably linked to a CAG promoter and a cytochrome c oxidase subunit VIII signal sequence, wherein the GFP is specifically expressed in mitochondria.

2. An isolated tissue or cell derived from the transgenic mouse of claim 1.

3. A screening method for an inducer of apoptosis, comprising the steps of:
   (a) contacting the transgenic mouse of claim 1 or the tissue or cell of claim 2 with a test substance; and
   (b) confirming whether or not apoptosis is induced on the basis of dynamics of mitochondria visualized by GFP.

4. A screening method for an apoptosis suppressor, comprising the steps of:
   (a) contacting the transgenic mouse of claim 1, or the tissue or cell of claim 2 with a test substance;
   (b) treating the mouse or the tissue or cell with the apoptosis inducer; and
   (c) confirming whether or not apoptosis is suppressed on the basis of dynamics of mitochondria visualized by GFP.

5. A method for producing a transgenic mouse whose genome comprises GFP expression specifically in the mitochondria, comprising the steps of:
   (a) introducing an expression vector comprising a GFP gene operably linked to a CAG promoter and a cytochrome c oxidase subunit VIII signal sequence into totipotent cells;
   (b) transferring the totipotent cells obtained in step (a) to the oviduct or uterus of a pseudo-pregnant mouse;
   (c) generating progeny from the pseudo-pregnant mouse of step (b);
   (d) selecting the transgenic strains, which have GFP transgene expression and fluorescence in isolated tissue or cell samples.

6. The transgenic mouse of claim 1, wherein the expression vector comprises the DNA fragment shown in SEQ ID NO:9.